

The Effect of Adding Sodium-Potassium and Calcium-Magnesium to The Diet of Native Chicken Hen on Sex Ratio of Native Chicks

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Abstract. Thirtysix native hens were used to determine the effect of adding Sodium-Potassium and Calcium-Magnesium to the diet of Native Chicken hens on determination of native chick sexes. Fifteen hens (n=5; control, Na-K and Ca-Mg) were fed for 1 month and the other hens (n=6, for control, Na-K and Ca-Mg) were fed for two months, then all hens were artificially inseminated, intra-vaginally, dose of 100 million perm cell per 0.1 ml, once a week for two weeks. On day 2 of insemination, all eggs were collected and evaluated daily for 14 days. Every 5 days, the selected eggs were incubated for 7 days and followed by candling to assess fertility. On day 21-22 of incubation, day old chicks were evaluated using vent sexing method to assess the sex ratio. Type of nutrition, fed at both for 1 month and two months had a similar results in fertility, hatchability and chick sex ratio: 62.79, 58.03, 59.52; 52.02, 50.93, 55.54 and 46.11, 51.89, 46.67 percent, respectively. Feeding period of three type of diet value for fertility, hatchability and sex ratio is 59.19, 61.04; 53.61, 52.05, and 48.24, 47.86, respectively. Our results suggest that different amount of ions in the diet of hens could not have a significant effect on the percent fertility, hatchability and sex ratio of chicks.

Key Words : Na-K, Ca-Mg, fertility, hatchability, sex ratio, chicks

Introduction

In general, gender of animal offspring is quite important to livestock producers. Because in beef cattle and sheep breeds, for example, the male grows at a faster rate than the female and hence is preferred for meat production, while the dairy farmer has little use for most bull calves, the use of sexed semen to produce only females would make milk production more efficient. In poultry, egg-produced farmers would choose hens because only hens will produce eggs (Garner, 2001).

A sperm from the male or an egg from the female contains one of each pair of autosomes; in addition, in mammals the egg always contains an X chromosome, while the sperm always carries either an X or Y chromosome. When a sperm and egg unite and the sperm carries the Y chromosome, the offspring is male (XY); however, if the sperm carries an X chromosome when it unites with the egg, the resulting offspring is female (XX) (Pike and Petrie 2003; Kaleta and Redmann, 2008; Jimenez et al., 2003).

In most animals, including humans, the ratio of males to females is 50:50. Because the

determination of sex, or gender, takes place when a sperm fertilizes an egg, pre selection of gender by selecting the sperm that fertilize eggs must be done before the sperm are used for insemination (Li WM et al., 2008; Hollingsworth, 2005; Jongbloet, 2004).

In all bird species, including turkeys and chickens, the female determines the sex of offspring. In birds, the sex chromosomes are exactly the same in all sperm, so poultry sperm cannot be manipulated to preselect the sex of offspring (Smith and Sinclair, 2001; Berlin and Ellergren, 2004).

According to Joshi method, the four basic elements in diet i.e. sodium, potassium, calcium and magnesium two months before the planned conception, followed by a specific time period for intercourse in the target cycle results in around 97% success rate in achieving the pregnancy of the preferred sex (Vahidi and Sheikhha, 2007). The method is totally safe, non invasive, economical and safely done at home with once a month consultation or correspondence based guidance by the doctor. The method is devoid of any side effects. Moreover dr Joshi said that gender diet can change the pH level in the body, and therefore,

it can change the polarity of the egg. The resulted charge will attract one gender more than the other 80 %. The increase in sodium and potassium is associated with increase in male birth, however for a girl, women need to eat food rich in calcium and magnesium. Experiments on mouse showed that pregnant female house mice maintained on a consistent low food diet (low Na-K) give birth to a lower proportion of males than do control females fed *adlibitum*. (Meikle and Thornton, 1995; Rosenfeld and Roberts, 2004). However, there are no studies to indicate the adding sodium-potassium and calcium-magnesium to the hen diet to change the sex ratio of native chicks. The aim of this study was to assess whether the use of sodium-potassium and calcium-magnesium given to the hen diet would alter the sex ratio towards males and females of the chicken offspring.

Materials and Methods

A total of 4 native (kampong) roosters along with 36 native hens were used in this study at Poultry research station, experimental farm, Animal Science Faculty, University of Jenderal Soedirman, Purwokerto. The fowls were housed in individual battery cages. Clean drinking water was provided to roosters and hens at all times. The male received 150 g/d of a diet containing 2,400 kcal ME/kg and 140 g/kg crude protein. Hens were randomly divided into three groups of 6 hens each.

Group one (D_1) was chosen as a control group, Group two (D_2) was received the diet mixed with 1% sodium-potassium, and Group three (D_3) consumed diet mixed with 1% calcium-magnesium. All the hens received 110 g of a diet containing 2800 kcal ME/kg and 170 g/kg crude protein. The males were 18 months of age and the female were 8 months of age at the start of the experiment.

Semen was routinely collected two times a week by abdominal massage (Saleh DM and Sugiyanto, 2006; Lake and Stewart, 1978) and used without any selection according to quantity or quality parameters of semen production.

All hens were artificially inseminated (100 million sperm cells/0.1 ml) once a week for two weeks. AI was performed between 1500 and

1600 hours at ambient temperature via deep-intra-vaginal (3-4 cm into the vagina) insemination using a tuberculin syringe.

On day 2 of insemination, all eggs were collected daily for 14 days. Every 5 days, the selected eggs were incubated for 7 days and followed by candling to assess fertility. On day 21-22 of incubation, day old chicks were evaluated using vent sexing method to assess the sex ratio.

This experiment was laid out in 3x2 factorial design, in which mineral diet (D_1 = control, D_2 = Na-K diet and D_3 = Ca-Mg diet) was considered as a first factor and consumption period (P_1 = 1 month and P_2 = 2 months) as the second factor. Native hens were allocated to the six groups ($n=6$).

After 30 days (group P_1) and 60 days (P_2), the hens were artificially inseminated once a week for 2 weeks.

Measurements

1. Fertility test. Sperm concentrations were estimated with hemacytometer. The final concentrations of ready to use artificial insemination doses ranged from 100 to 125 x 10⁹ spermatozoa per milliliter. A total of 6 native hens per treatment were inseminated once a week for a total of two intra-vaginal inseminations (100 million sperm per hen). Artificial inseminations were performed at 3 cm depth with a one-ml tuberculin syringe. The fertilizing ability of sperm cells was estimated from eggs collected on Days 2 to 7 after each insemination of fresh semen. Fertility rates (fertilized eggs/incubated eggs x 100) were determined by candling eggs on day 7 of incubation, and questionable eggs were broken out to investigate early embryonic development. Any questionable eggs were classified as infertile. After 18 days of incubation, the remaining eggs were candled again, and dead embryos were referred to as the second.

2. Hatchability. Hatchability data were recorded after 22 days of incubation, recording pipped eggs, dead chicks. Hatchability as the ratio of hatched to fertilized egg x 100.

3. Sex ratio. Sex ratio of chicks was observed by vent sexing is performed by examining a day old chick's vent for the presence, or lack of, the formation of a male sex organ. The chick is held

upside down to perform the examination and questionable of chick sexes were kept them grow. The easiest but the longest way to tell sex is to simply watch the chickens grow. In males, look for a larger comb, shinier, more pointed tail feathers and crowing. Females generally have smaller combs, tail feathers that are more rounded on the ends and of course, they lay eggs. Sex ratio of male to female (male chicks/hatched eggs \times 100) (Klein and Grossman, 2008; Bull et al., 2007; Kemsley, 2008).

Statistical Analysis

Data were analyzed by analysis of variance using randomized completely block design for 3x2 factorial design. Fertility and Hatchability data were transformed to arc sin $\sqrt{\%}$ before analysis. Differences between treatment means were assessed by the least significant difference test (Steel and Torrie, 1983).

Results and Discussion

The total average of fertility was 60.11 ± 1.72 percent. The highest fertility rates were obtained with control hens (62.79 ± 1.33 %, Table 1). However, The lowest fertility rates were obtained with hens received Na-K (58.03 ± 0.50). However, the mineral treatments yielded fertility rates (58.03 to 62.79%) that was not significantly different. Our results suggest that different amount of ions in the diet of native hens could not have significant effect of the fertility, and moreover to increase the fertility of hens, the artificial insemination should be performed maximal at 4-day interval.

The average of hatchability was $52.83 \pm 1.39\%$, ranged from 50.93 to 55.54%. The highest fertility rates were obtained with the hens received Ca-Mg diet, and the lowest

fertility rates were achieved by hens received Na-K diet. However, statistical analysis showed that the hatchability was not affected by mineral diets. The hatchability yielded in this experiment was lower than result reported by some researchers (Islam et al., 2002; Al-Daraji, 2001). The cause of low hatchability may be caused by lack of incubation management.

Effect of adding mineral and consumption period prior hens inseminated on sex ratio of native chicks is presented in Table 1.

The average sex ratio value was 48.05 ± 2.05 percent. In group Na-K, under sodium and potassium diet, 12 hens (72 fertilized eggs, 38 day old chicks). Their gender was 48.05% male and 51.95% female chicks. In group Ca-Mg under calcium and magnesium diet, all of the 12 hens (75 fertilized eggs, 40 day old chicks. Finally, in the control group all of the 12 hens, 120 eggs and hatched of 39 chicks. The difference between the sex offspring between group Na-K and Ca-Mg was not statistically significant. This results is not agreed with experiments results in rat and sow that if mineral imbalance in the diet of the female before fertilization affect the sex ratio of the progeny, out of a total of 677 births, the sex ratio was 55.7 with the sodium and potassium diet 48.3 with the calcium and magnesium diet (Bolet et al., 1982; Vahidi and Seikhha, 2007). These results show that sodium and potassium diet and is in favour of male birth, which is not similar to our results.

Conclusions

Our study showed that percent fertility, hatchability and sex ratio of the native chicks were not affected by adding mineral Na-K and Ca-Mg to hen diet for 1-2 months.

Table 1. Fertility, hatchability and chick sex ratio in kedu hens fed with various mineral diet for one and two months

Measurements	One month feeding period			Two months feeding period			Level of significance		
	Control	NaK	CaMg	Control	NaK	CaMg	Mineral	Period	Interaction
Fertility	61.44	57.53	58.57	58.57	58.53	60.46	NS	NS	NS
Hatchability	55.67	50.96	54.20	48.37	50.89	56.08	NS	NS	NS
Sex ratio	46.67	47.22	50.83	45.55	55.55	42.50	NS	NS	NS

NS : Non significant difference ($P > 0.05$)

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References

- Al-Daraji HJ. 2001. Sperm-egg penetration in laying breeder flocks: a technique for the prediction of fertility. *British Poultry Sci.* 42:266-270.
- Berlin S and H Ellergren. 2004. Chicken W : A genetically uniform chromosome in a highly variable genome. *Proc. of the National Academy of Sciences of the United States of America.*
- Bolet G, L Gueguen, P Dando and L Oliver. 1982. Influence of mineral diet of the sow on the sex ratio of the new born. *Reprod. Nutr. Dev.* 22:1073-1081.
- Garner DL, 2001. Sex-sorting mammalian sperm: concept to application in animals. *J. of Andrology* 22:4, July/August.
- Hollingsworth LD, 2005. Ethical considerations in prenatal sex.. *Health soc. Work*, 30:126-134.
- Islam MS, MAR Howlader, F Kabir and J Alam. 2002. Comparative assessment of fertility and hatchability of Barred Plymouth Rock, White Leghorn, Rhode Island Red and White Rock hen. *International J. Poultry Sci.* 1: 85-90.
- Jimenez A, R Fernandez, N Madrid-Bury, PN Moreira, C Borque and B Pintado. 2003. Experimental demonstraton that pre- and post-conceptional mechanisms influence sex ratio in mouse embryos. *Mol. Reprod. Dev.* 66:162-165.
- Jongbloet PH. 2004. Over-ripeness ovopathy. A chalenging hypothesis for sex ratio modulation. *Hum. Reprod*, 19:769-774.
- Kaleta EF and T Redmann. 2008. Approaches to determine the sex prior to and after incubation of chicken eggs and of day-old chicks. *World's Poultry Sci. J.* 64:391-399.
- Kemsley M, 2008. How to Guide Sexing Chicks. The State of Queensland, Australia.
- Klein S and R Grossman, 2008. Primary sex ratio in fertilized chicken eggs pends on reproductive age and selection. *J. Exp. Zoology Part A Ecol. Genet. Physiol.* I(1):35-46.
- Lake PE and JM Stewart. 1978. Artificial insemination in poultry. *Bulletin* 213. Her Majesty's Stationary Office. London.
- Li WM, YP Feng, RX Zhao, YZ Fan, NA Affara, JJ Wu, J Fang, Q Tng, C Wang and SJ Zhang. 2008. Sex ratio bias in early-dead embryos of chickens collected during the first week of incubation. *J. Poultry Sci.* 87:2231-2233.
- Meikle DB and MW Thornto. 1995. Premating and gestational effects of maternal nutrition on secondary sex ratio in house mice. *J. Reprod. Fertility* 105:193-196.
- Pike TW and M Petrie. 2003. Potential mechanisms of avian sex manipulation. *Biological Reviews* 78:553-574.
- Rosenfeld CS and RM Roberts. 2004. Maternal diet and other factors affecting offspring sex ratio; A Review. *Biol. of Repro.* 71:1063-1070.
- Saleh DM and Sugiyatno. 2006. Effect of Insemination time on fertility of kampung rooster sperm. *J. of Anim. Prod.* 8(2):83-87.
- Sheikhha MH and AR Vahidi. 2007. The Effects of Sodium, Potassium, Calcium and Magnesium on the Ratio of Male to Female Offspring of Vistar Rats. *Pakistan J. of Nut.* 6(1)44-48.
- Smith CA and AH Sinclair 2001. Sex determination in the chicken embryo. *J. Exp. Zool.* 1:290(7):691-699.
- Steel RGC and JH Torrie 1983. *Principles and Procedures of Statistics.* McGraw-Hill Book Co., Inc., New York.
- Vahidi AR and MH Sheikhha. 2007. Comparing the effects of iodium and potassium diet with calcium and magnesium diet on sex ratio of rat' offspring. *Pakistan J. of Nut.* 6(1):44-48.